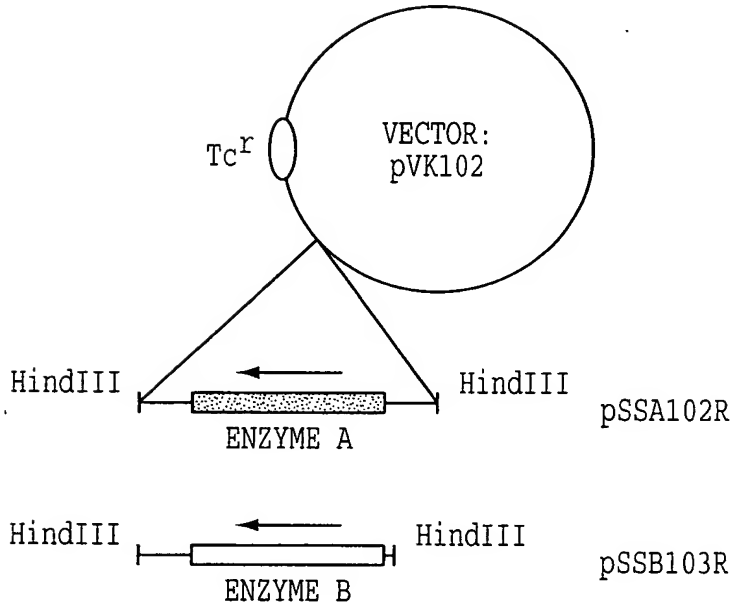

Tc^r
VECTOR:
pVK102
HindIII
HindIII
pSSA102R
ENZYME A
HindIII
HindIII
pSSB103R
ENZYME B

FIG. 1

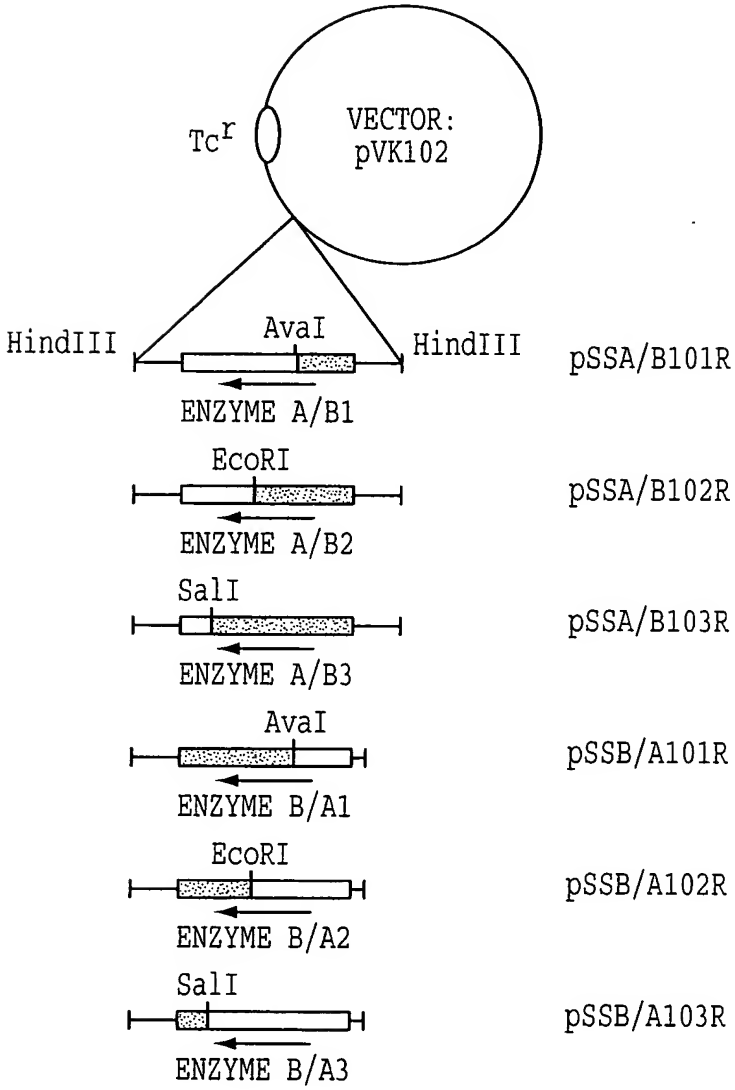
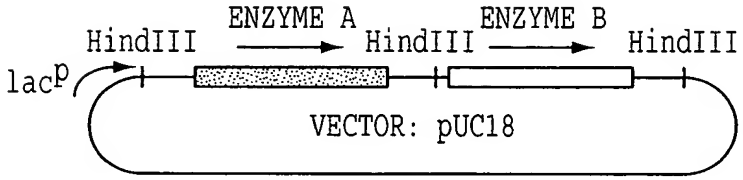

Tc^r
VECTOR:
pVK102
HindIII
AvaI
HindIII
pSSA/B101R
ENZYME A/B1
EcoRI
pSSA/B102R
ENZYME A/B2
SalI
pSSA/B103R
ENZYME A/B3
AvaI
pSSB/A101R
ENZYME B/A1
EcoRI
pSSB/A102R
ENZYME B/A2
SalI
pSSB/A103R
ENZYME B/A3

FIG. 2

pSSAB201



pSSBA201

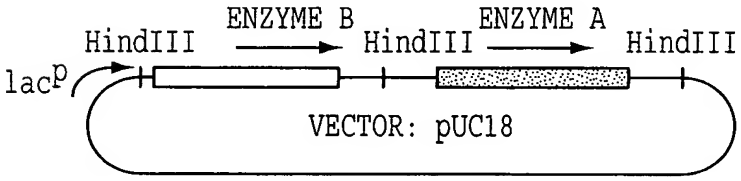
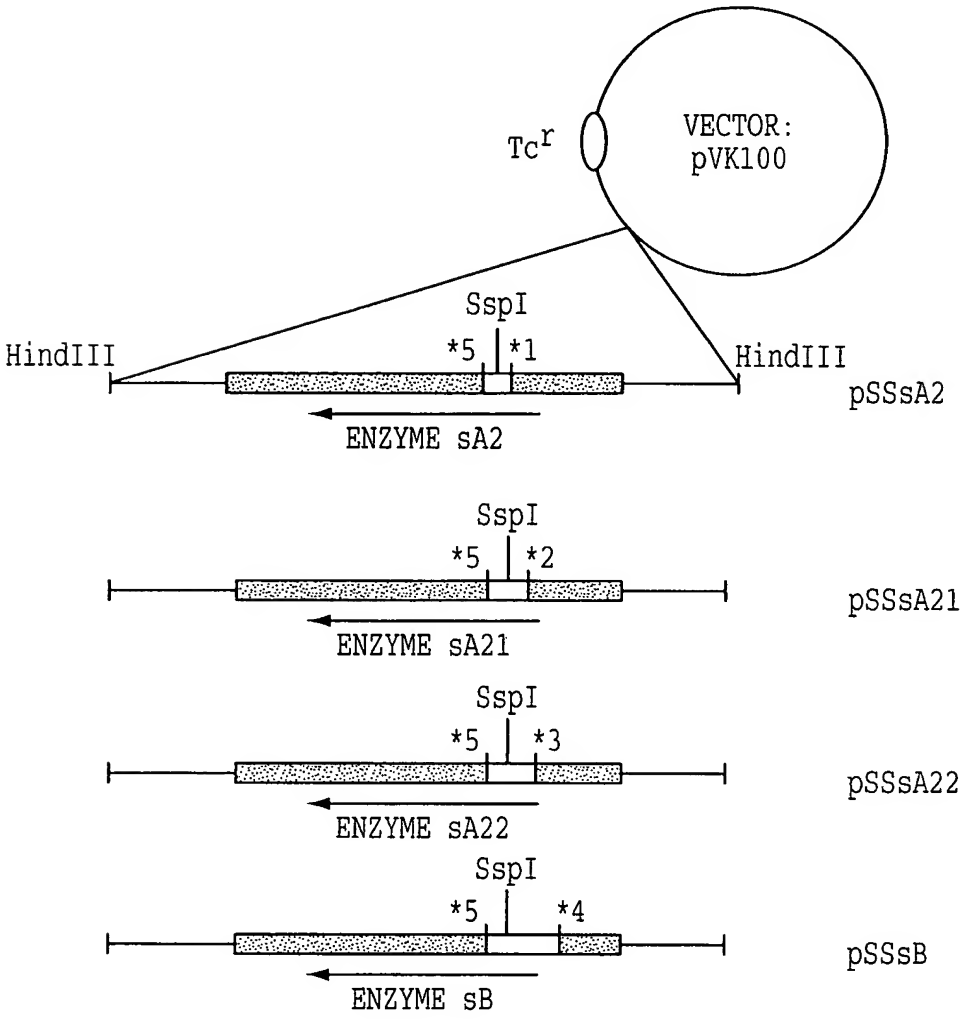


FIG. 3



RECOMBINATION SITE

*1 : AMINO ACID RESIDUE NO. 135 OF MATURE ENZYME A

*2 : AMINO ACID RESIDUE NO. 128 OF MATURE ENZYME A

*3 : AMINO ACID RESIDUE NO. 125 OF MATURE ENZYME A

*4 : AMINO ACID RESIDUE NO. 95 OF MATURE ENZYME A

*5 : AMINO ACID RESIDUE NO. 180 OF MATURE ENZYME B, WHICH NUCLEOTIDE SEQUENCE OF AvaI SITE ENCODES

FIG. 4

```
ENZYME A 1 : QVTPVTDELL ANPPAGEWIS YGQNQENYRH SPLTQITTEN VGQLQLVWAR GMQPGKVQVT
             **** ***** *********  ** ******* *******  * ********** **  * ****
ENZYME B 1 : QVTPITDELL ANPPAGEWIN YGRNQENYRH SPLTQITADN VGQLQLVWAR GMEAGAVQVT

          61 : PLIHDGVMYL ANPGDVIQAI DAKTGDLIWE HRRQLPNIAT LNSFGEPTRG MALYGTNVYF
               * ******** *********  ** ******* ******  ** **  *   **  *****  **
          61 : PMIHDGVMYL ANPGDVIQAL DAQTGDLIWE HRRQLPAVAT LNAQGDRKRG VALYGTSLYF
                                                                              *
                                                                           AvaI
         121 : VSWDNHLVAL DTATGQVTFD VDRGQGED-M VSNSSGPIVA NGVIVAGSTC QYSPFGCFVS
                ****** ** *  **** ** * ** ***    **  ***** ********** **** *** *
         121 : SSWDNHLIAL DMETGQVVFD VERGSGEDGL TSNTTGPIVA NGVIVAGSTC QYSPYGCFIS

         180 : GHDSATGEEL WRNYFIPRAG EEGDETWGND YEARWMTGAW GQITYDPVTN LVHYGSTAVG
               ********** *** ***  * **********  ******* * ********** ** **** **
         181 : GHDSATGEEL WRNHFIPQPG EEGDETWGND FEARWMTGVW GQITYDPVTN LVFYGSTGVG

         240 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVTNVDVQPS
               ********** ********** ********** ********** ********** ** *******
         241 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVANVDVQPS
                                                          *
                                                      EcoRI
         300 : TEMEGLQSIN PNAATGERRV LTGVPCKTGT MWQFDAETGE FLWARDTNYQ NMIESIDENG
                *****  ** ********** *** ****** ** ***  ** *********  *** **** *
         301 : AEMEGLRAIN PNAATGERRV LTGAPCKTGT MWSFDAASGE FLWARDTNYT NMIASIDETG

         360 : IVTVNEDAIL KELDVEYDVC PTFLGGRDWP SAALNPDSGI YFIPLNNVCY DMMAVDQEFT
               ******* * ********** *********  ******* ** ** **** ** * *******
         361 : LVTVNEDAVL KELDVEYDVC PTFLGGRDWS SAALNPDTGI YFLPLNNACY DIMAVDQEFS
                                                                    *
                                                                  SalI
         420 : SMDVYNTSNV TKLPPGKDMI GRIDAIDIST GRTLWSVERA AANYSPVLST GGGVLFNGGT
                 ******    ** **     ********** ****** **  **********  *** *****
         421 : ALDVYNTSAT AKLAPGFENM GRIDAIDIST GRTLWSAERP AANYSPVLST AGGVVFNGGT

         480 : DRYFRALSQE TGETLWQTRL ATVASGQAIS YEVDGMQYVA IAGGGVSYGS GLNSALAGER
               ********** ******* ** **** ***** ** ** ** * *  **  **   **  ** *
         481 : DRYFRALSQE TGETLWQARL ATVATGQAIS YELDGVQYIA IGAGGLTYGT QLNAPLA-EA

         540 : VDSTAIGNAV YVFALPQ
                ***  ***  *******
         540 : IDSTSVGNAI YVFALPQ
```

* : NUCLEOTIDE SEQUENCES ENCODING THESE REGIONS ARE THE RESTRICTION SITES FOR AvaI, EcoRI, AND SalI WHICH WERE USED FOR CONSTRUCTING CHIMERA GENES SHOWN IN FIG. 2.

FIG. 5

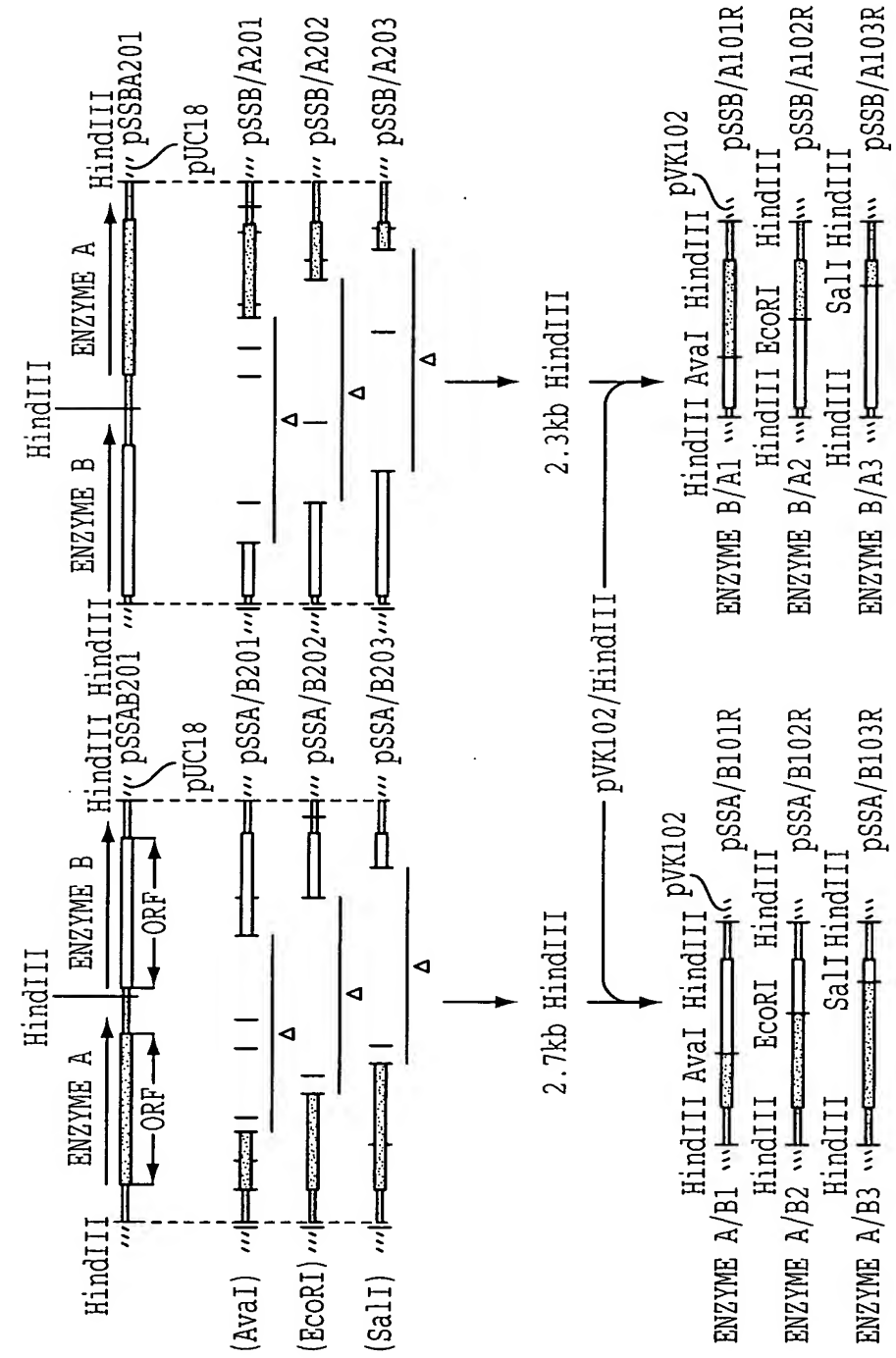
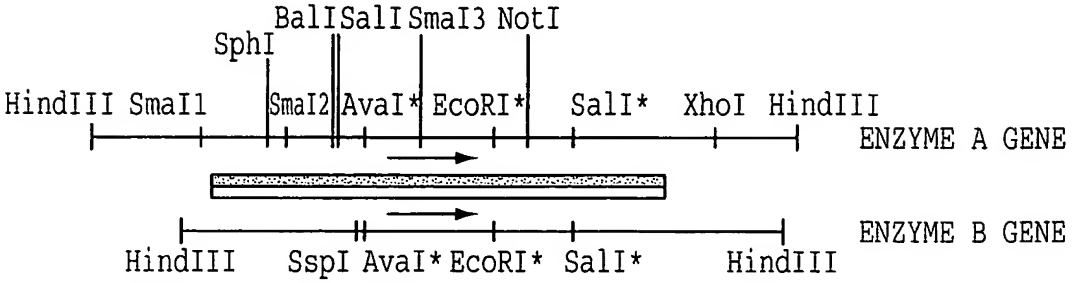

HindIII
ENZYME A
ENZYME B
HindIII
pSSBA201
pUC18
ORF
HindIII HindIII
pSSAB201
pUC18
(AvaI)
(EcoRI)
(SalI)
pSSA/B201
pSSA/B202
pSSA/B203
pSSB/A201
pSSB/A202
pSSB/A203
2.7kb HindIII
2.3kb HindIII
pVK102/HindIII
pVK102
HindIII AvaI HindIII
HindIII EcoRI HindIII
HindIII SalI HindIII
ENZYME A/B1
ENZYME A/B2
ENZYME A/B3
pSSA/B101R
pSSA/B102R
pSSA/B103R
ENZYME B/A1
ENZYME B/A2
ENZYME B/A3
pSSB/A101R
pSSB/A102R
pSSB/A103R

FIG. 6



*: AvaI, EcoRI, SalI SITES USED FOR CONSTRUCTING CHIMERA GENES SHOWN IN FIG. 2 AND 6.

FIG. 7

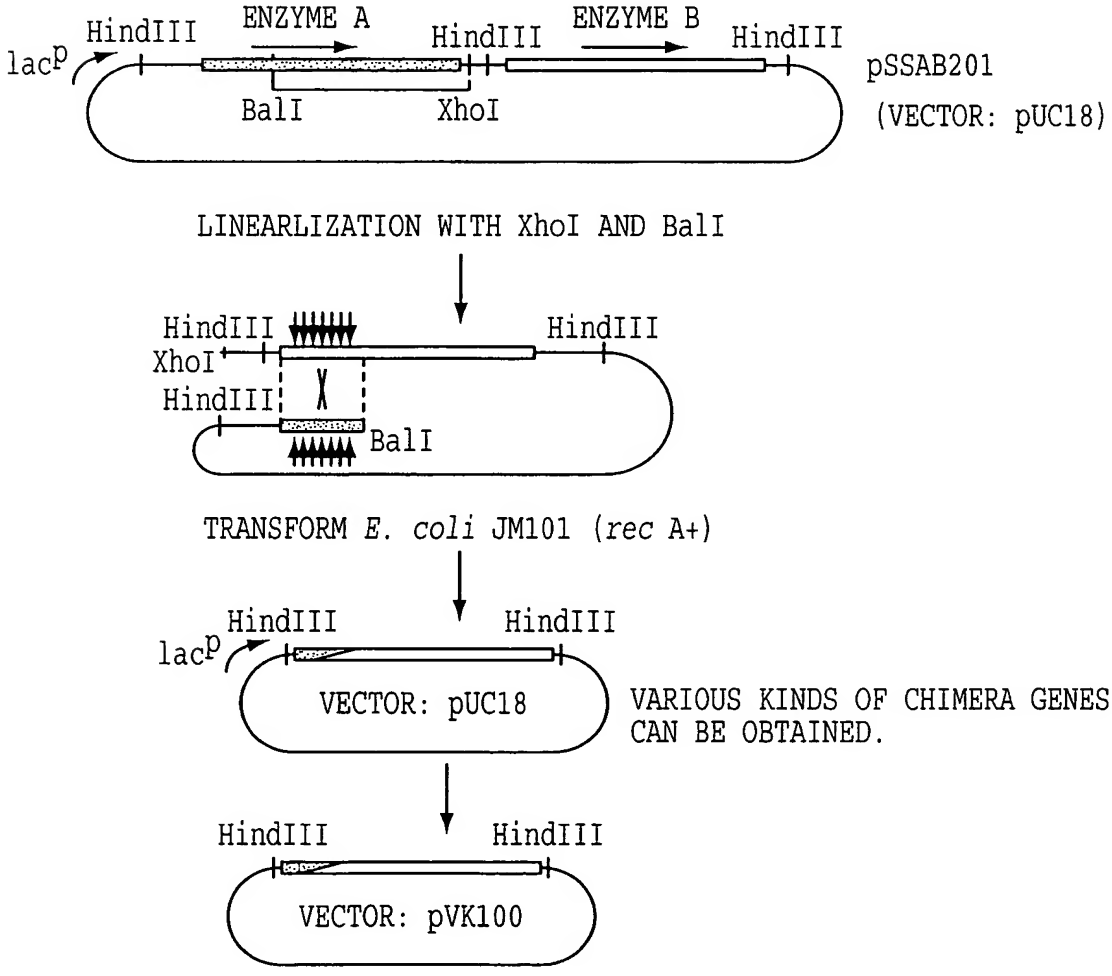


FIG. 8

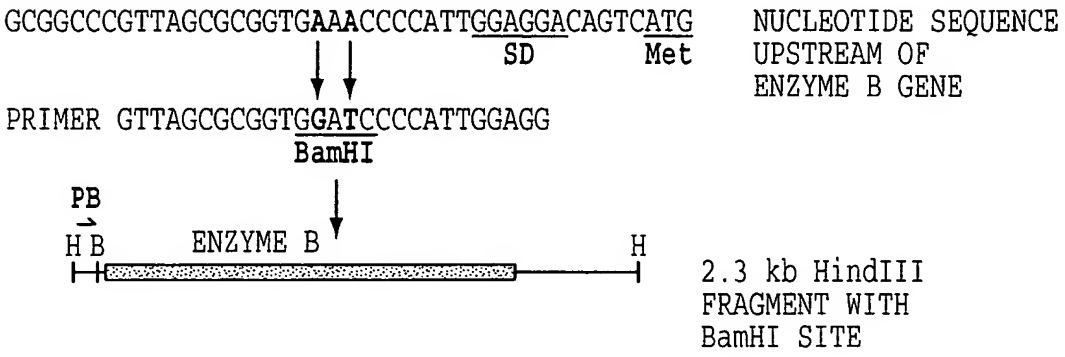

GCGGCCCGTTAGCGCGGTGAAACCCCATTGGAGGACAGTCATG
SD
Met
NUCLEOTIDE SEQUENCE UPSTREAM OF ENZYME B GENE
PRIMER GTTAGCGCGGTGGATCCCCATTGGAGG
BamHI
PB
H B
ENZYME B
H
2.3 kb HindIII FRAGMENT WITH BamHI SITE

FIG. 9

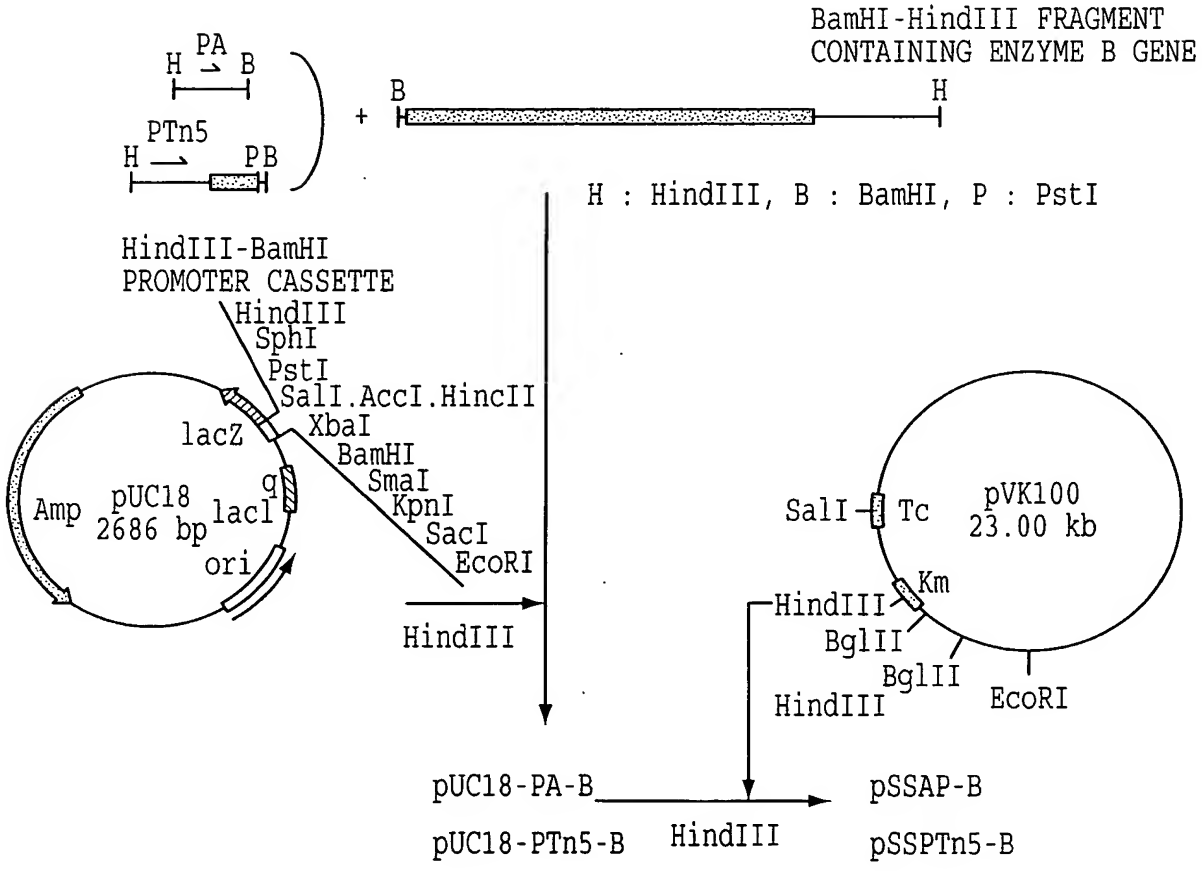
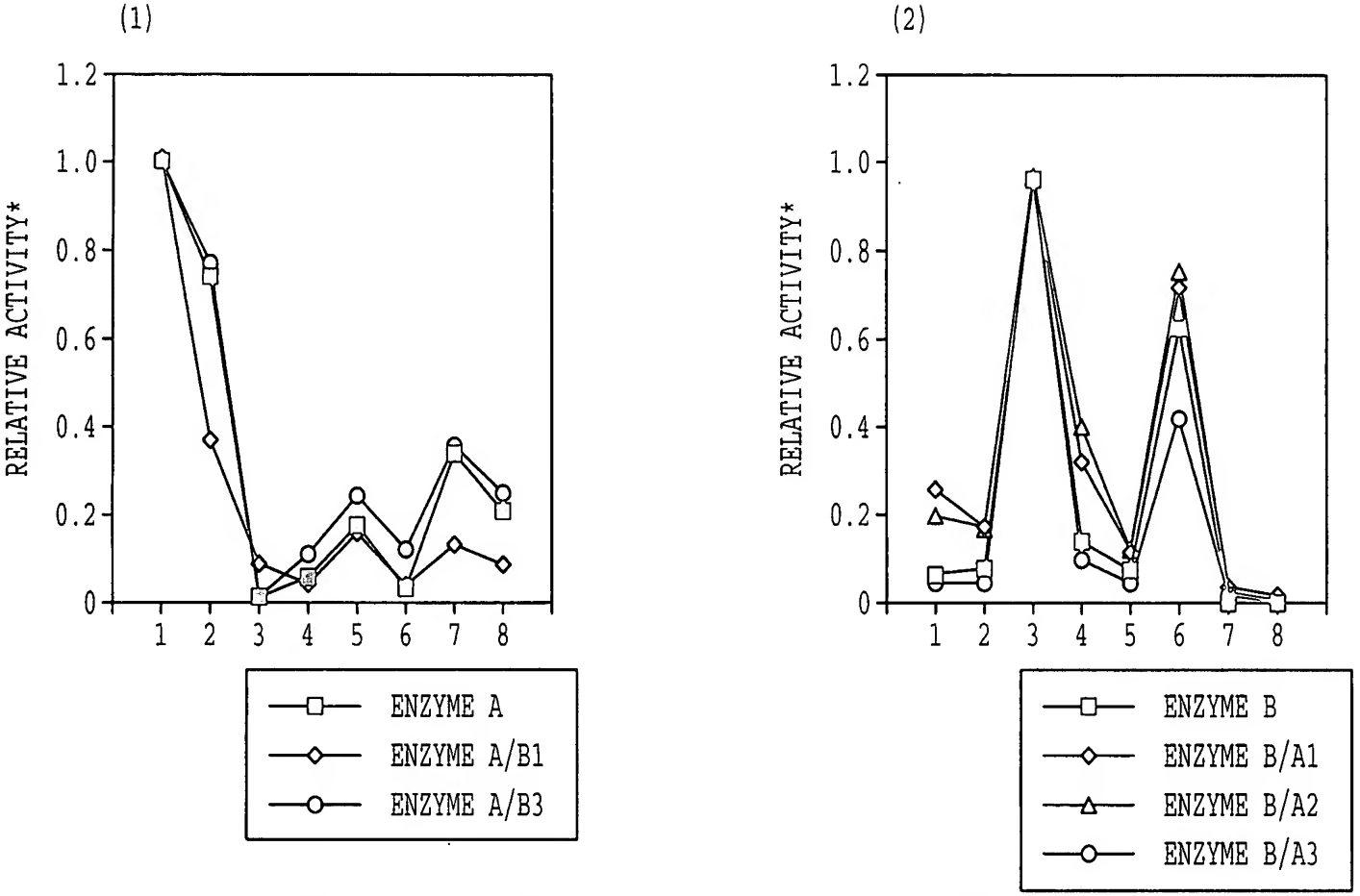

BamHI-HindIII FRAGMENT CONTAINING ENZYME B GENE
PA
H B
PTn5
H PB
B
H
H : HindIII, B : BamHI, P : PstI
HindIII-BamHI PROMOTER CASSETTE
HindIII
SphI
PstI
SalI.AccI.HincII
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
lacZ
Amp
pUC18 2686 bp
lacI
q
ori
HindIII
SalI
Tc
pVK100 23.00 kb
Km
HindIII
BglII
BglII
HindIII
EcoRI
pUC18-PA-B
pUC18-PTn5-B
HindIII
pSSAP-B
pSSPTn5-B

FIG. 10



1. n-PROPANOL, 2. ISOPROPANOL, 3. D-GLUCOSE, 4. L-SORBOSONE
5. D-SORBITOL, 6. D-MANNITOL, 7. L-SORBOSE, 8. D-FRUCTOSE

*ENZYME ACTIVITY WAS NORMALIZED RELATIVE TO ACTIVITY FOR n-PROPANOL (1), OR D-GLUCOSE (2). ENZYME A/B2 WAS EXCEPTED BECAUSE OF ITS LOW EXPRESSION IN *P. putida*.

FIG. 11